



A new species of frog (Terrarana, Strabomantidae, *Phrynopus*) from the Peruvian Andean Grasslands

Germán Chávez^{1,2}, Luis A. García-Ayachi^{1,2}, Alessandro Catenazzi^{1,2,3}

¹ Instituto Peruano de Herpetología (IPH), Augusto Salazar Bondy 136, Urb. Higuiereta, Surco, Lima, Perú

² Centro de Ornitología y Biodiversidad (CORBIDI), Lima, Perú

³ Department of Biological Sciences, Florida International University, Miami, FL, USA

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Corresponding author: Germán Chávez (vampflack@yahoo.com)

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Abstract

We describe a new terrestrial frog from the puna grasslands adjacent to the Mantaro dry valley in southern Peru. *Phrynopus apumantarum* sp. nov. is similar in appearance to *P. bufoides* but is differentiable by lacking discoidal fold and enlarged warts on dorsum, lacking a prominent post ocular fold and having canthal and post ocular stripe. Lastly, we propose to place the new species under the Vulnerable (VU) category of the IUCN Red List, due its small distribution and habitat loss recorded at the type locality.

Key Words

new terrestrial frog, southern Peru, *Phrynopus apumantarum* sp. nov., Vulnerable, Red List, habitat loss

Introduction

Terrestrial-breeding frogs of the genus *Phrynopus* Peters, 1873 inhabit the high Andean puna grasslands and montane forests in central and northern Peru (Fig. 1; Lehr et al. 2012). The overall body shape of *Phrynopus* and similar frogs in related genera (*Lynchius* Hedges, Duellman & Heinicke, 2008, *Oreobates* Jiménez de la Espada, 1872, and even *Pristimantis* Jiménez de la Espada, 1870) presumably converged in response to the conditions of these high elevation environments (Padial et al. 2014; Rodriguez and Catenazzi 2017; Chávez et al. 2020). No species are known south of Provincia Concepción, in the Department of Junín (11°44'45"S, 74°58'47"W), central Peru, where *P. chaparroi* Mamani & Malqui, 2014 lives and is currently the most austral record for the genus.

To reach its current configuration, the Andean mountains have experienced multiple uplifts events since the Miocene (Gregory-Wodzicki 2000, cited by von May et al. 2018), which included volcano eruptions and earthquakes, resulting in an altitude gradient that heavily influenced the climatic conditions, habitats and, subsequently the mor-

phology of the organisms that inhabited these mountains. Geographical isolation promoted vicariance processes which are recognized as one of the causes of Andean vertebrate diversification and endemism (Duellman 1999; Wiens et al. 2007; Santos et al. 2009; Benham and Witt 2016; Hazzi et al. 2018). Mountain top isolation could also explain why *Phrynopus*, a group of frogs with scarce locomotion skills and low vagility (Chávez et al. 2020) is, with 35 species, one of the most diverse genera of Andean amphibians (Frost 2023). The distribution of *Phrynopus* frogs goes north to south, from the eastern Andean slopes of northern Peru (type locality of *P. mariellaleo* Venegas, Barboza, De la Riva & Padial 2018) to the Andean grasslands of Junin, southeastern Peru (type locality of *P. chaparroi* Mamani & Malqui 2014). Most of the species are distributed in the eastern and central Andes, with the only exception of *P. thompsoni* Duellman 2000 that inhabits the highlands of the western Andes in northern Peru.

In southern Peru, we find a mix of isolated mountains and Inter-Andean dry Valleys associated with the Mantaro and Apurimac River basins (Fig. 1). The arid conditions of these dry valleys are possibly a barrier and cause of vicariance for

species living in the adjacent wet highlands. Certainly, the hard conditions and rough terrain of these mountains have made the Mantaro River Basin hard to reach by scientific expeditions. Thus, is not surprising that herpetological surveys carried out in the last decade have resulted in the discovery of new species: one water frog, *Telmatobius mantaro* (Ttito et al. 2016), and the lizards *Ameiva reticulata* (Landauro et al. 2015), *Stenocercus diploauris* (Venegas et al. 2020), and *S. nigrobarbatus* (Venegas et al. 2020).

As a result of multiple expeditions, we obtained several specimens of *Phrynopus* from the highlands of the Mantaro River basin in the Peruvian Andes. Detailed external revision of the specimens revealed unique combinations of morphological features not found in any other described species of *Phrynopus*. Furthermore, genetic analyses led us to confirm that our specimens from the field corresponded to an undescribed species. Here we present the results of our work and we describe the new species.

Materials and methods

For format of description, we follow Lynch and Duellman (1997), Rodriguez and Catenazzi (2017) and Lehr et al. (2017), as well as character definitions given by Duellman and Lehr (2009). We followed Hedges et al. (2008) and Heinicke et al. (2017) for family placement. We collected specimens during the day while conducting visual transect surveys. We euthanized specimens with benzocaine 20%, preserved them in 10% formalin, and stored them in 70% alcohol in the Herpetology Collection of Centro de Ornitología y Biodiversidad (**CORBIDI**). We used a digital caliper under a microscope to measure the following to the nearest 0.1 mm: snout-vent length (**SVL**, from the tip of the nose to cloaca), tibia length (**TL**), foot length (**FL**, distance from proximal margin of inner metatarsal tubercle to tip of toe IV), head length (**HL**, from angle of jaw to tip of snout), head width (**HW**, at level of angle of jaw), eye diameter (**ED**), interorbital distance (**IOD**), upper eyelid width (**EW**), internarial distance (**IND**), and eye to nostril distance (E-N, straight line distance between anterior corner of orbit and posterior margin of external nares). We numbered fingers and toes preaxially to post axially from I–IV and I–V respectively. We determined comparative lengths of toes III and V by adpressing both toes against toe IV; lengths of fingers I and II were determined by adpressing the fingers against each other. Specimens were sexed based on external sexual characteristics (e.g., the presence of vocal sacs) or through dissections to the evaluation of gonads. To reduce reflections, preserved holotypes were photographed submerged in ethanol. Photographs taken in the field by the authors were used for descriptions of colour in life. We obtained information on species for comparative diagnoses from Duellman and Lehr (2009) and from original species descriptions. For specimens examined see Appendix 1.

We conducted phylogenetic analyses to confirm generic placement of the new species, and to examine their

evolutionary relationships with other species of *Phrynopus*. We relied on newly generated sequences from our specimen CORBIDI 20438, and sequences available in GenBank for species of *Phrynopus* and related genera *Niceforonia* Goin & Cochran, 1963, *Lynchi*us, and *Oreobates* (Appendix II: table A1; Padial et al. 2014; De la Riva et al. 2017; von May et al. 2018). We analyzed fragments of five genes, including the three mitochondrial genes 12S, 16S, and the protein-coding gene cytochrome c oxidase subunit I (**COI**), and the two nuclear genes recombination-activating protein 1 (**RAG1**) and Tyrosinase precursor (Tyr), using the same primers and thermocycling conditions during the polymerase chain reaction of von May et al. (2018). We followed standard protocols (Hedges et al. 2008) for extraction, amplification, and sequencing of DNA. We ran the polymerase chain reaction (**PCR**) with a ProFlex thermal cycler (Applied Biosystems). We purified PCR products with Exosap-IT (Affymetrix, Santa Clara, CA, USA), and shipped purified samples to MCLAB (San Francisco, CA, USA) for sequencing. We aligned sequences using Geneious R11, version 11.1.5 (Biomatters, <http://www.geneious.com/>) with the MAFFT v7.017 alignment program (Katoh and Standley 2013), and trimmed sequences to a length of 387, 520, 630, 628, and 483=2648 bp for 12S, 16S, COI, RAG, and Tyr respectively.

Table 1. Measurements and proportions of the type series of *Phrynopus apumantarum* sp. nov. Ranges values followed by average and standard deviation (in parentheses).

	<i>Phrynopus apumantarum</i> sp. nov.	
	Males (n=6)	Females (n=10)
SVL	19.3–25.7 (23.5 ± 2.2)	27.7–35.7 (31.4 ± 2.9)
HL	5.5–7.9 (7.1 ± 0.8)	8.2–10.3 (9.1 ± 0.6)
HW	7.7–9.2 (8.6 ± 0.5)	10.1–12.6 (11.5 ± 0.9)
ED	1.7–2.5 (2.1 ± 0.2)	2.3–3 (2.6 ± 0.2)
EW	1.9–2.3 (2.1 ± 0.1)	2–3.2 (2.4 ± 0.3)
IOD	2–3.1 (2.6 ± 0.3)	2.2–4 (3 ± 0.5)
IND	2–2.4 (2.2 ± 0.1)	2.4–3.2 (2.8 ± 0.2)
E-N	1.3–1.8 (1.5 ± 0.2)	1.8–2.2 (2 ± 0.1)
FL	7.1–9.3 (8.4 ± 0.7)	9.7–12.7 (11.4 ± 1)
TL	6.4–8.4 (7.7 ± 0.6)	9.1–11.4 (10.2 ± 0.8)
HL/SVL	0.2–0.3 (0.3 ± 0)	0.2–0.3 (0.2 ± 0)
HW/SVL	0.3–0.4 (0.3 ± 0)	0.3–0.3 (0.3 ± 0)
HW/HL	1.1–1.4 (1.2 ± 0)	1.1–1.3 (1.2 ± 0)
FL/SVL	0.3–0.3 (0.3 ± 0)	0.3–0.3 (0.3 ± 0)
TL/SVL	0.3–0.3 (0.3 ± 0)	0.2–0.3 (0.3 ± 0)

We conducted an analysis using Maximum Likelihood with IQ-TREE v1.6.12 (Nguyen et al. 2015) for phylogenetic inference of the concatenated sequence of the five gene fragments. We used PartitionFinder software version 1.1.1 to select the best partitioning scheme and substitution model for each gene. Our analysis included 38 terminals and a 2,648-bp alignment for the partitioned dataset, branch length was unlinked among the partitions. We used the same partition scheme of von May et al. (2018), with six subsets as follows (each set corresponding to a number from 1 to 6): (1) model GTR + I + G for

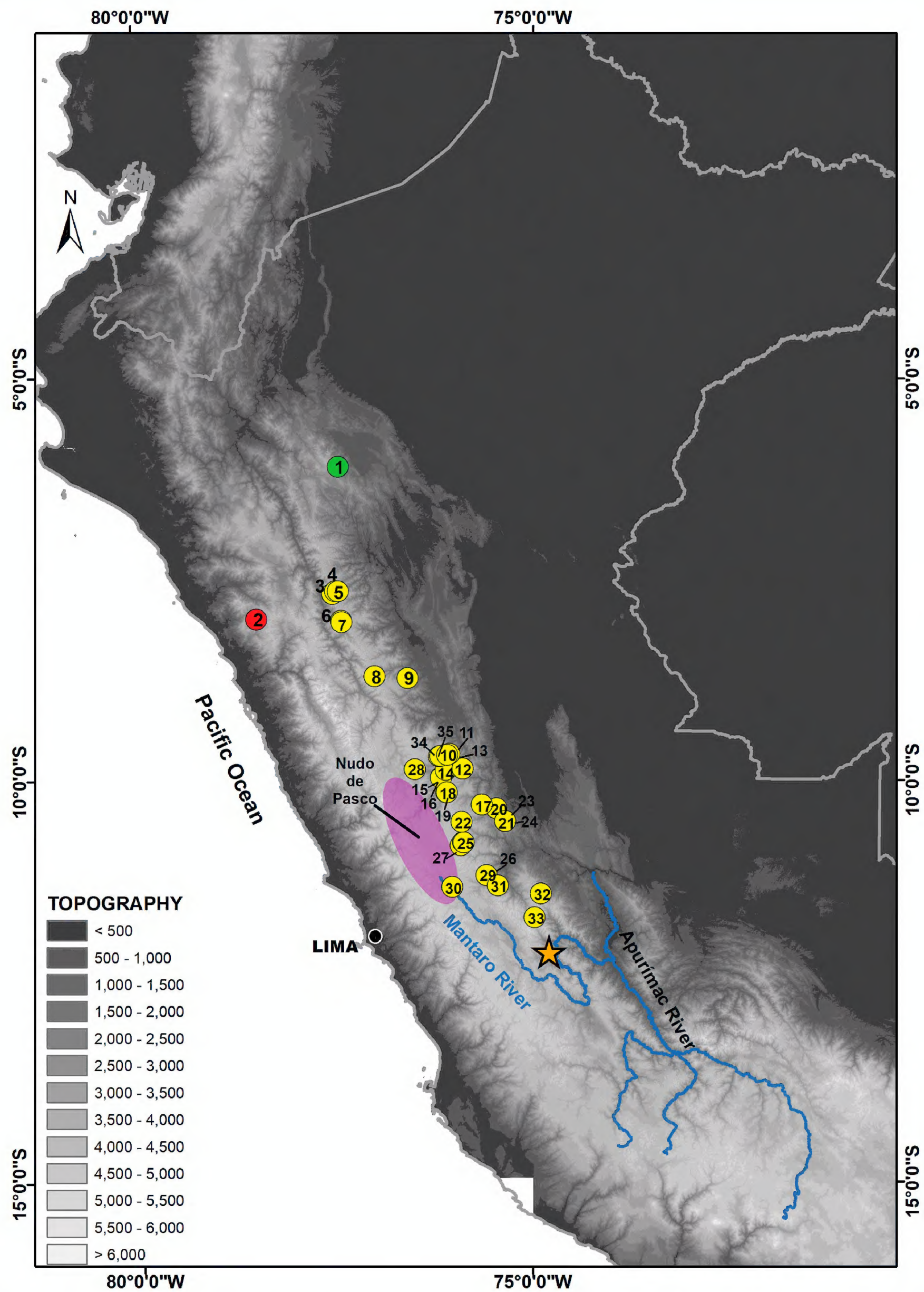


Figure 1. Map of the distribution of *Phrynopus* frogs. Type locality of *P. apumantarum* sp. nov. in orange star. Green circle: distribution area of *P. mariellaleo*; Red circle: distribution area of *P. thompsoni*; yellow circles show distribution area of: 1) *Phrynopus mariellaleo*, 2) *P. thompsoni*, 3) *P. capitalis*, 4) *P. dumicola*, 5) *P. personatus*, 6) *P. anancites*, 7) *P. valquii*, 8) *P. remotum*, 9) *P. daemon*, 10) *P. vestigiatus*, 11) *P. lechriorhynchus*, 12) *P. kauneorum*, 13) *P. dagmarae*, 14) *P. interstinctus*, 15) *P. horstpauli*, 16) *P. heimorum*, 17) *P. mirosławae*, 18) *P. tautzorum*, 19) *P. barthlenae*, 20) *P. badius*, 21) *P. tribulosus*, 22) *P. pesantesi*, 23) *P. auriculatus*, 24) *P. bracki*, 25) *P. paucari*, 26) *P. juninensis*, 27) *P. bufoides*, 28) *P. kotosh*, 29) *P. montium*, 30) *P. peruanus*, 31) *P. oblivious*, 32) *P. inti*, 33) *P. chaparroi*, 34) *P. lapidoides*, 35) *P. unchog*.

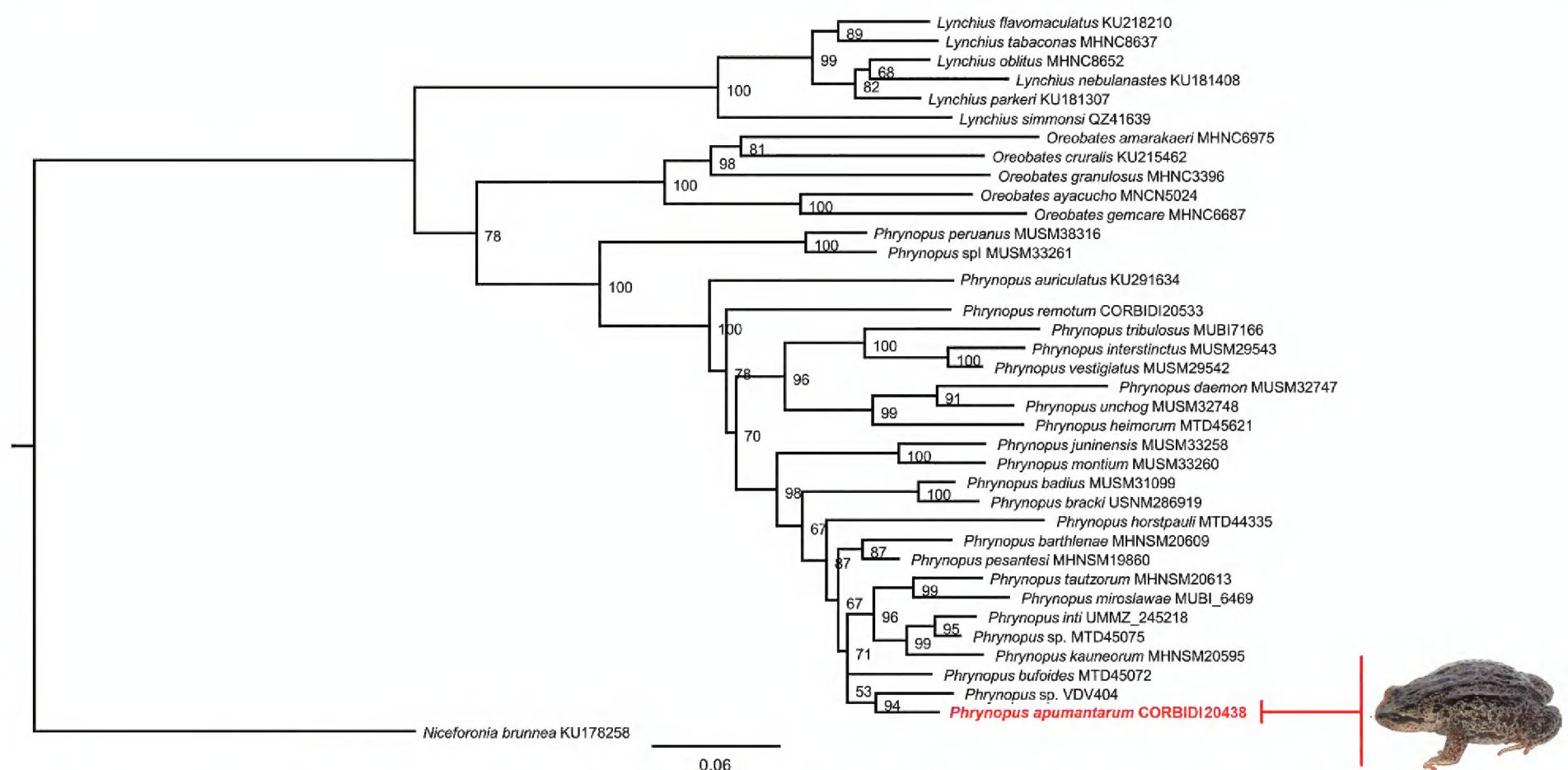


Figure 2. Phylogeny of *Phrynopus* inferred with a Maximum Likelihood approach. Maximum Likelihood tree for *Lynchius*, *Niceforonia*, *Oreobates* and *Phrynopus* species included in this study based on a 2,648-bp concatenated dataset of gene fragment of 12S and 16S rRNA, cytochrome c oxidase subunit 1, recombination-activating protein 1, and tyrosinase precursor, analyzed in IQTREE (posterior probabilities are indicated at each node). The new species *P. apumantarum* is in red.

sequences 16S and 12S, (2) model K80 + I for 1st codon position of COI, (3) HKY + I for 2nd codon position of COI, (4) GTR + G for 3rd codon position of COI, (5) HKY + I for 1st and 2nd codon positions of RAG1 and Tyr, and (6) HKY + G for 3rd codon position of RAG1 and Tyr. In IQ-TREE, we used the ultrafast bootstrap method (10000 bootstrap alignments).

We also estimated uncorrected p-distances (i.e., the proportion of nucleotide sites at which any two sequences are different) for 16S sequences of a larger number of species in MEGA X (Kumar et al. 2018). The reason this analysis is restricted to 16S is that it is the most commonly sequenced gene for *Phrynopus* (Appendix 2). We uploaded the table of p-distances to Figshare (<https://doi.org/10.6084/m9.figshare.20947858>).

Nomenclatural act

The electronic version of this article in Portable Document Format (PDF) will represent a published work according to the International Commission on Zoological Nomenclature (ICZN), and hence the new name contained in the electronic version is effectively published under that Code from the electronic edition alone. This published work and its nomenclatural acts have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed through any standard web browser by appending the LSID to the prefix <http://zoobank.org/>. The LSID for this publication is: urn:lsid:zoobank.org:pub: DD30F5F2-65D2-40A1-BF75-C064D504F7E1.

Results

Generic assignment

Although there currently is no known synapomorphic phenotypic trait for *Phrynopus* that allows to distinguish species in this genus from other Terrarana with similar body shape and anatomical structures, our molecular phylogeny (Fig. 2) clearly places the new species within the *Phrynopus* clade. According to our phylogeny, and to 16S p-distances, the new species is most closely related to an undescribed species from Ayacucho (voucher VDV404), and to a group of species composed by *P. bufoides* Lehr, Lundberg & Aguilar, 2005, *P. inti* Lehr, von May, Moravec & Cusi, 2017, *P. kauneorum* Lehr, Aguilar & Köhler, 2002, *P. mirosławae* Chaparro, Padial, and De la Riva, 2008 and *P. tautzorom* Lehr & Aguilar, 2003. Therefore, based on the results of our molecular analyses, we assign the new species to *Phrynopus*.

Taxonomy

Phrynopus apumantarum sp. nov.

<https://zoobank.org/926E068E-E965-4092-AE8E-E9F2FFC77083>

Figs 3A–E, 4A–H

Type material. Holotype. PERU • Adult female; Huanavelica Department, Tayacaja Province, the vicinity of San Luis de Rabayoc; 12°10'36.65"S, 74°48'2.49"W; 3715 m; 19 Jun. 2019; L.A. García-Ayachi leg.; CORBIDI 20553 (Figs 3A–E, 4C, D).

Paratopotypes. PERU • 3 ♀ adults, 4 ♂ adults, collected on 22 Feb. 2019 at the same place as holotype;

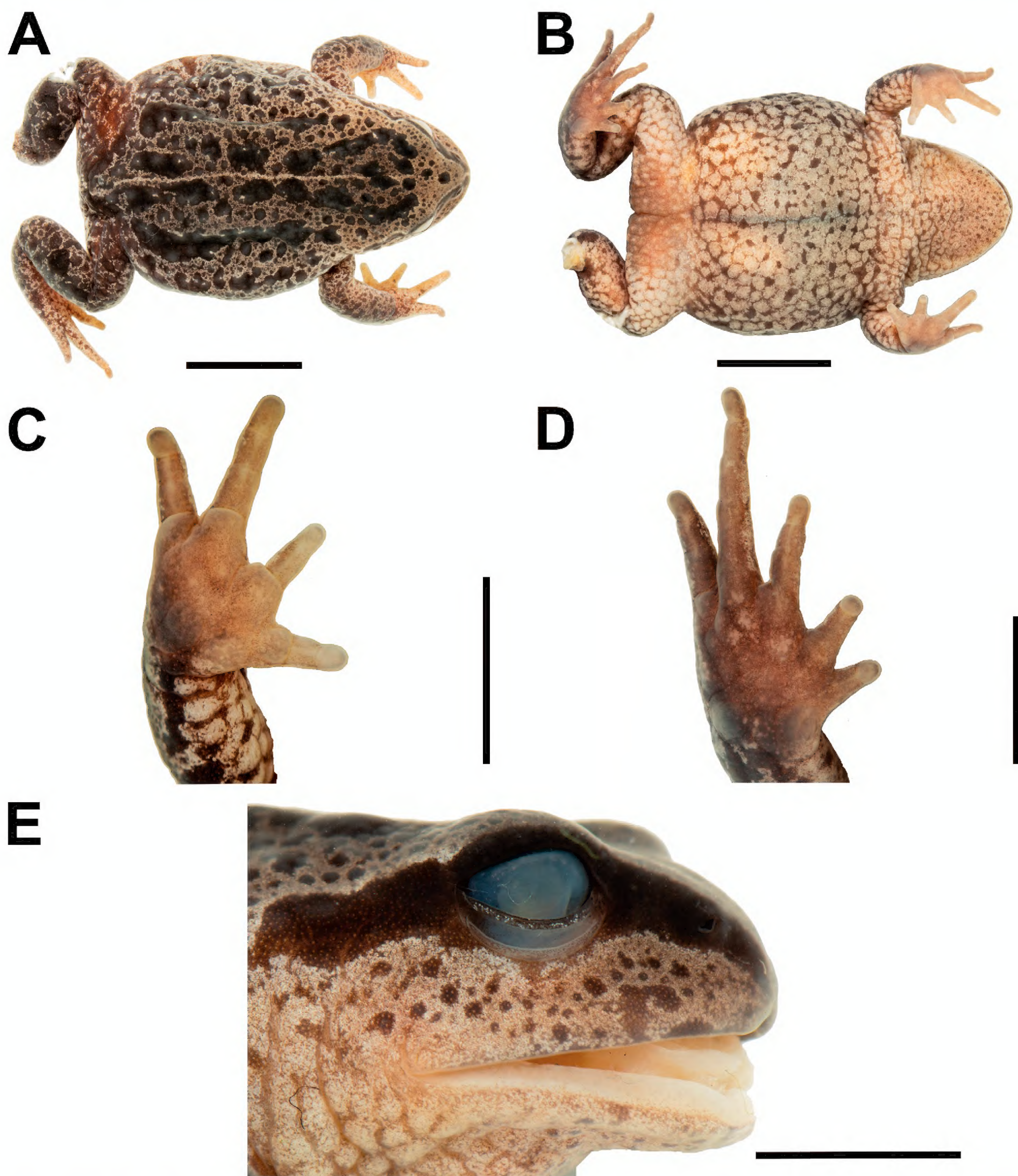


Figure 3. Holotype of *Phrynopus apumantarum* sp. nov. (CORBIDI 20553). **A.** Dorsal view of the body; **B.** Ventral view of the body; **C.** Ventral view of the right hand; **D.** ventral view of the right foot; **E.** Lateral view of the head. Scale bar: 10 mm (A–D); 5 mm (E).

L.A. García-Ayachi leg.; CORBIDI 20432–35, 20436–39 (Fig. 4A, B, E–H) • 5 ♀ adults, 2 ♂ adults, collected with the holotype; CORBIDI 20551, 20555–58, 20552, 20554.

Diagnosis. A species of *Phrynopus* having the following combination of characters: 1) Skin on dorsum coarsely tuberculate, some tubercles enlarged and arranged longitudinally in paravertebral and dorsolateral rows, skin on venter coarsely areolate; discoidal fold absent, thoracic fold absent; postocular fold present, incomplete

dorsolateral ridges present; 2) tympanic membrane and tympanic annulus absent; 3) snout rounded in dorsal and lateral views; 4) upper eyelid with small rounded tubercles; narrower than IOD, cranial crests absent; 5) dentigerous process of vomers absent; 6) vocal slits and nuptial pads absent; 7) Finger I slightly shorter than finger II; tips of digit bulbous, rounded; 8) fingers without lateral fringes; 9) ulnar and tarsal tubercles absent; 10) heels without tubercles; 11) inner metatarsal tubercle rounded, about

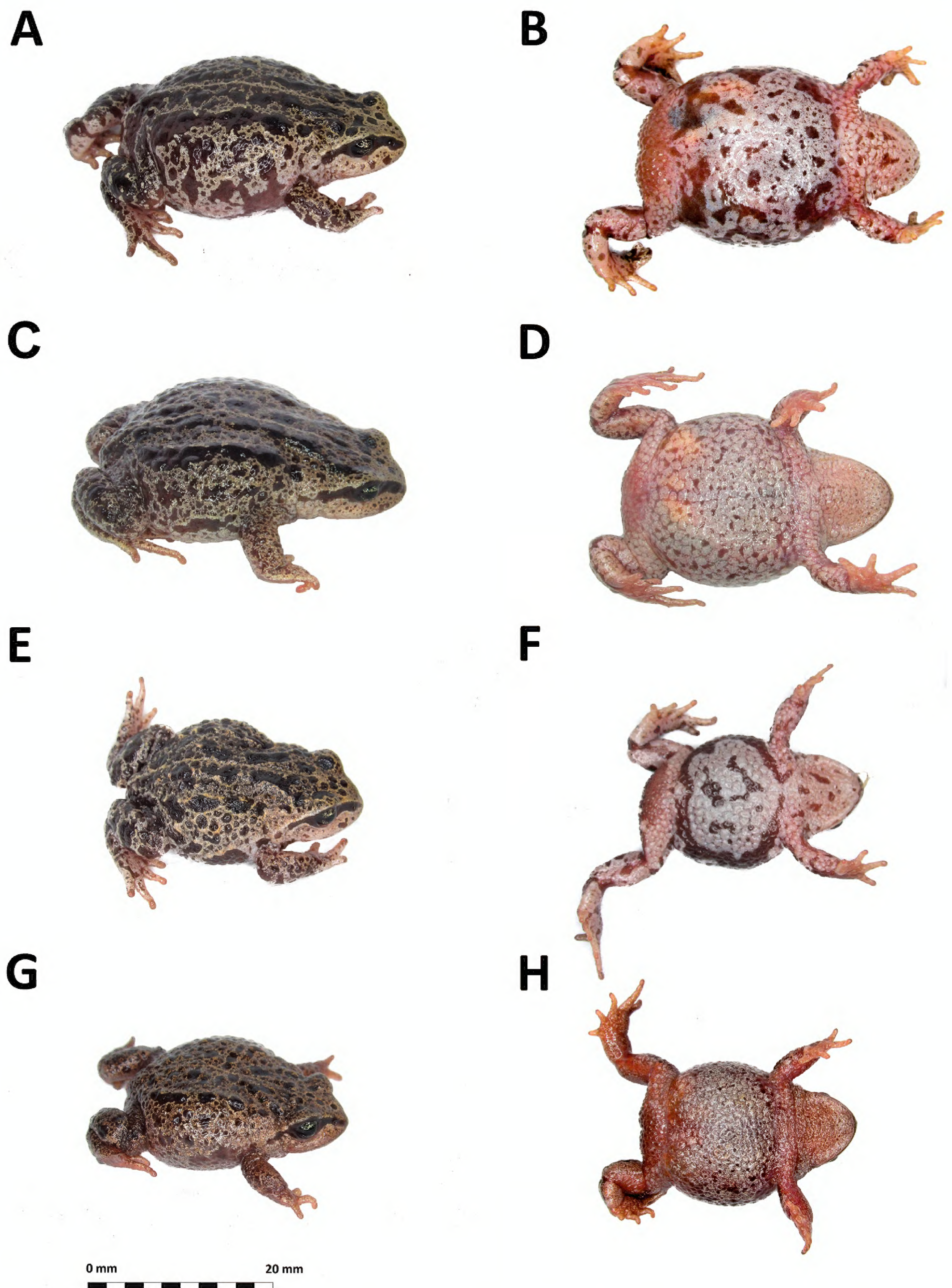


Figure 4. Type series of *Phrynopus apumantarum* sp. nov. Dorsal (left column) and ventral (right column) view of the type series of *Phrynopus apumantarum* sp. nov. **A, B.** Female CORBIDI 20432; **C, D.** Female CORBIDI 20553 (holotype); **E, F.** Male CORBIDI 20438; **G, H.** Male CORBIDI 20439.

1.5 times as large as ovoid outer metatarsal tubercle; supernumerary plantar tubercles absent; 12) toes without lateral fringes, basal webbing absent, toe V slightly longer than toe III, toe tips bulbous; 13) in life, dorsum grayish yellow with black or dark brown mottling and bluish-black or brown blotches, throat pinkish yellow, yellow or yellowish brown, venter grayish yellow with brown mottling and bluish-black blotches, groins grayish white with brown blotches; iris ash gray with black blotches and reticulations; (14) SVL 23.9–25.7 mm in males ($n = 4$), and 28.4–35.7 mm in females ($n = 4$).

Comparisons. The absence of tympanic membrane and tympanic annulus distinguishes *Phrynopus apumantarum* sp. nov. from *P. auriculatus* Duellman & Hedges, 2008, *P. mariellaleo* Venegas, Barboza, De la Riva & Padial, 2018 and *P. peruanus* Peters, 1873, the only three species in the genus with tympanic membrane and tympanic annulus. Also, *P. apumantarum* sp. nov. resembles externally to *P. bufoides*, but is easily distinguishable by lacking the discoidal fold (vs present), warts on dorsum absent (vs enlarged warts present), post ocular fold moderately developed (vs prominent), and by having dark canthal and post ocular stripe (vs absent). Furthermore, the dark mottling or blotches on dorsum makes *P. apumantarum* sp. nov. similar in appearance to *P. barthlenae* Lehr & Aguilar, 2002, *P. horstpauli* Lehr, Köhler & Ponce, 2000, *P. mirosławae*, *P. tautzorum*, and *P. thompsoni* Duellman, 2000. However, *P. apumantarum* is distinguished from them by the following characters (morphological features of the other of species in parenthesis): presence of a postorbital fold (vs absent in *P. barthlenae*, *P. horstpauli*, *P. tautzorum*, and *P. thompsoni*), rounded tubercles on the upper eyelid (vs absent in *P. horstpauli*, *P. tautzorum*, and *P. thompsoni*), having skin on venter coarsely areolate (vs areolate in *P. barthlenae*, *P. horstpauli*, *P. mirosławae*, and *P. tautzorum*), lacking discoidal and thoracic fold (vs at least one of them present in *P. barthlenae*, *P. horstpauli*, *P. mirosławae*, and *P. thompsoni*), and absence of tubercles on heels (vs present in *P. barthlenae* and *P. mirosławae*). Only *P. chaparroi* from the other side of the Mantaro River dry valley (51.6 km airline from the type locality of the new species) and *P. remotum* Chávez, García-Ayachi & Catenazzi, 2020 from the northern Andes of Peru share with *P. apumantarum* sp. nov. a combination of characters consisting of head rounded, small tubercles on the upper eyelid, skin on venter areolate and the absence of discoidal and thoracic fold, however the latter could be differentiated by having a bigger size with a maximum SVL in females of 35.7 mm (vs 32.2 mm in *P. chaparroi*, and 28.3 mm in *P. remotum*), canthal and postorbital stripes present (vs absent in *P. chaparroi*) a dorsum coarsely tuberculate (vs shagreen with small subconical tubercles in *P. remotum*), tubercles on dorsum arranged longitudinally (vs scattered in all dorsum in *P. chaparroi* and *P. remotum*), and heels without tubercles (vs rounded tubercles on heels

in *P. remotum*). Furthermore, *P. apumantarum* sp. nov. is similar to *P. anancites* Rodríguez & Catenazzi, 2017, *P. capitalis* Rodríguez & Catenazzi, 2017, and *P. lapidoides* Lehr & Rodríguez, 2017 in having a coarsely areolate or areolate skin on venter, however is differentiable from all of them by bearing tubercles on dorsum arranged paravertebrally and dorsolaterally. Also *P. apumantarum* has small rounded tubercles on upper eyelid (vs absent in *P. anancites* and *P. capitalis*), skin on dorsum coarsely tuberculate (vs tuberculate in *P. capitalis* and *P. lapidoides*), discoidal and thoracic folds absent (vs at least one of them present in *P. anancites*, *P. capitalis*, and *P. lapidoides*), and Toe V slightly longer than toe III (vs toe V longer than toe III in *P. capitalis*). Despite having a combination of dorsal tubercles arranged paravertebrally as well as dorsolaterally and skin on venter coarsely areolate makes *P. apumantarum* sp. nov. clearly distinct from the rest of congeners, we consider that *P. badius* Lehr, Moravec & Cusi, 2012, *P. curator* Lehr, Moravec & Cusi, 2012, *P. daemon* Chávez, Santa Cruz, Rodríguez & Lehr, 2015, *P. dagmarae* Lehr, Aguilar & Köhler, 2002, *P. dumaticola* Rodríguez & Catenazzi, 2017, *P. heimorum* Lehr, 2001, *P. kotosh* Lehr, 2007, *P. montium* (Shreve, 1938), *P. paucari* Lehr, Lundberg & Aguilar, 2005, *P. pesantesi* Lehr, Lundberg & Aguilar, 2005, *P. unchog* Lehr & Rodríguez, 2017, and *P. vestigiatus* Lehr & Oróz, 2012 which are species bearing dorsal ridges (or folds) and/or an areolate venter might not easily distinguished from the new species. Nevertheless, *P. apumantarum* sp. nov. can be differentiated by having a bigger size with females reaching SVL of 35.7 mm (vs 18.8–28.3 mm in *P. badius*, *P. curator*, *P. daemon*, *P. dagmarae*, *P. dumaticola*, *P. heimorum*, *P. kotosh*, *P. montium*, *P. paucari*, *P. unchog*, and *P. vestigiatus*), discoidal fold absent (vs present in *P. paucari*), thoracic fold absent (vs present in *P. badius*, *P. curator*, *P. daemon*, *P. dagmarae*, *P. dumaticola*, *P. heimorum*, *P. kotosh*, and *P. unchog*), canthal stripe present (vs absent in *P. daemon*, *P. dumaticola*, *P. kotosh*, *P. paucari*, *P. unchog*, and *P. vestigiatus*), postorbital stripe present (vs absent in *P. daemon*, *P. kotosh*, *P. paucari*, *P. pesantesi* and *P. unchog*), postocular fold present (vs absent in *P. dagmarae*, *P. kotosh*, *P. heimorum*, and *P. paucari*), tubercles on heels absent (vs present in *P. dagmarae* and *P. vestigiatus*), and toe V slightly longer than toe III (vs toe V shorter than toe III in *P. dumaticola*; toe V slightly shorter than toe III in *P. daemon* and *P. heimorum*). The rest of the species (*P. bracki* Hedges, 1990, *P. inti* Lehr, von May, Moravec & Cusi, 2017, *P. interstinctus* Lehr & Oróz, 2012, *P. juninensis* (Shreve, 1938), *P. kauneorum* Lehr, Aguilar & Köhler, 2002, *P. lechriorhynchus* Trueb & Lehr, 2008, *P. oblivious* Lehr, 2007, *P. personatus* Rodríguez & Catenazzi, 2017, *P. tribulosus* Duellman & Hedges, 2008, and *P. valquii* Chávez, Santa Cruz, Rodríguez & Lehr, 2015) lack areolate skin on venter and/or tubercles on dorsum.

Description of the holotype. Head as wide as body, wider than long, HW 130% of HL; HW 35% of SVL; HL 27% of SVL; snout short, rounded in dorsal and lateral views (Fig. 3A, E), ED larger than E-N distance (E-N 75% of ED); nostrils protuberant, directed dorsolaterally; canthus rostralis slightly curved in dorsal view, rounded in profile; loreal region slightly concave; lips rounded; upper eyelid with small rounded tubercles; UEW narrower than IOD (EW 72% of IOD); postocular folds low, poorly developed, extending from posterior margin of upper eyelid to level of tympanic region (Fig. 3B); tympanic membrane and tympanic annulus absent, tympanic region without postrictal tubercles. Choanae small, triangular, close to but not concealed by palatal shelf of maxilla; dentigerous processes of vomers absent; tongue broad, about 1.5 as long as wide, not notched posteriorly, posterior half free; vocal slits absent.

Skin on dorsum coarsely tuberculate with enlarged tubercles arranged paravertebrally and dorsolaterally (Fig. 3A); skin on flanks shagreen with scattered, low tubercles; skin on throat, chest and belly coarsely areolate (Fig. 3B); discoidal fold absent, thoracic fold absent; cloacal sheath not distinct; cloacal region without tubercles. Outer surface of forearm with small rounded tubercles; outer palmar tubercle barely visible, low, ovoid, slightly smaller than ovoid inner palmar tubercle; supernumerary tubercles absent; subarticular tubercles low, ovoid, most prominent on base of fingers; fingers without lateral fringes; Finger I shorter than Finger II (Fig. 3C); tips of digits rounded, bulbous, lacking circumferential grooves; nuptial pads absent.

Hind limbs long and robust, TL 31% of SVL; FL 37% of SVL; dorsal surface of hind limbs shagreen with scattered low tubercles; anterior surfaces of thighs shagreen with small few tubercles, posterior surfaces of thighs coarsely areolate; heel without tubercles; outer surface of tarsus without tubercles; outer metatarsal tubercle rounded and prominent, about as large as prominent ovoid inner metatarsal tubercle; supernumerary plantar tubercles absent; subarticular tubercles low, ovoid in dorsal view, most distinct on base of toes; toes without lateral fringes; basal webbing absent; toe tips bulbous, rounded, lacking circumferential grooves, about as large as those on fingers; relative lengths of toes: $1 < 2 < 3 < 5 < 4$; Toe V slightly longer than Toe III (Fig. 3C, D).

Measurements of the holotype (in mm). SVL 34.3; tibia length 10.8; foot length 12.7; head length 9.3; head width 12.2; eye diameter 2.9; interorbital distance 3.3; upper eyelid width 2.4; internarial distance 2.6; eye-nostril distance 2.2.

Coloration of the holotype in life (Fig. 4C, D). Dorsum greyish yellow with dark brown mottling and bluish-brown enlarged blotches. Flanks dark brown with greyish yellow reticulations. Canthal and postorbital stripes brown. Upper lip bearing brown irregular spots. Arms and legs dorsally dark brown with greyish yellow reticulations. Throat and chest greyish yellow with pinkish brown blotches, belly greyish yellow with dark brown

mottling. Groin, posterior surfaces of thighs, posterior surfaces of tibias, and dorsal surfaces of feet greyish white with brown blotches or spots. Iris ash grey with fine black reticulations.

Coloration of the holotype in preservative (Fig. 3A–E). Dorsum greyish cream with dark brown spots and blotches. Flanks the same color as dorsum, with brown mottling. Canthal and postorbital stripes dark brown. Upper lip with few pale brown spots. Arms and legs dorsally tan with dark brown blotches and spots. Groin greyish cream. Throat, chest, and venter creamy yellow and dark brown mottled. Ventral surfaces of hand and feet pinkish yellow. Iris bluish grey.

Variation. All paratypes are similar in morphology to the holotype, but males have slightly wider heads than females (HW/SVL in males = 0.3–0.4 vs females = 0.3), see Table 1 for variation in measurements and proportions. Main variations are noted on the dorsum coloration (Fig. 4A–H) which consists of a dark brown background with black spots and enlarged blotches in male CORBIDI 20436; or of an olive-yellow background with dark brown enlarged blotches on scapular and sacral areas, and black spots on the head in female CORBIDI 20433, 20434, and 20435, and of a brownish yellow background with dark brown spots or blotches in male CORBIDI 20439. Also, postorbital stripe is interrupted in female CORBIDI 20435 and male CORBIDI 20437. Supralabial region varies from dark brown in male CORBIDI 20436 to olive yellow in male CORBIDI 20438, and brownish yellow in male CORBIDI 20439. Regarding venter coloration, the throat is usually pinkish yellow, yellow in female CORBIDI 20434, and yellowish brown in male CORBIDI 20436. Also, the belly lacks large dark blotches in female CORBIDI 20439 and bears the largest dark blotches arranged at the edge of the venter in male CORBIDI 20437.

Etymology. The epithet *apumantarum* derives from the Quechua word *apu* (= mountain spirit), and from the name Mantaro which is the main river of the Valley where the new species was discovered. This name means the spirit of the Mantaro Mountains because the occurrence of the new species in the upper areas of that mountain ridge reminds the authors of the Inca legend that says Apus are always taking care of the Andes from the top of every valley in the region.

Distribution, Natural History, and Conservation status. *Phrynopus apumantarum* sp. nov. is known only from the type locality at 3714 m a.s.l. (Fig. 1). All the specimens were found under rocks and moss, during daytime (around 10:00 am), in an Andean relict patch of forest under rocks and moss, near a pasture area (Fig. 5). The vegetation consisted mainly of small bushes and epiphytes. No sympatric anurans were recorded during sampling, the only other vertebrate recorded was *Wilsonosaura josyi* which seemed to have diurnal habits, however, none of them was observed sharing the same stone with *P. apumantarum* sp. nov.



Figure 5. Landscape at the type locality of *P. apumantarum* sp. nov.

In nearby areas, local farmers use the land to grow potatoes which involves burning the ground seasonally, an activity that seems to increase in frequency and that threatens the habitat of this species. Therefore, given its apparent small distribution range (less than 2000 km² of occurrence), fragmented habitat, and the threats mentioned above which are further fragmenting the habitat at the type locality and nearby areas, we recommend the IUCN Red List Category VU 2ab (iii) for this species. Future field efforts in the area will be needed to confirm its persistence and the possible reduction of its population.

Discussion

Previous research has demonstrated that geographical barriers in mountain ecosystems could be the main factor explaining the current diversification and distribution of vertebrates in Andean regions (Winger and Bates 2015; Hazzi et al. 2018). Within this framework, it is important to consider that the Mantaro-Apurimac Dry Valley in southern Peru (Fig. 1) has been regarded as an important focus of isolation and subsequent diversification of amphibians, birds, and plants (Lehr and Catenazzi 2008; Sarkinen 2011; Hazzi et al. 2018; Linares-Palomino 2018). Likewise, the Mantaro-Apurimac dry valley has been considered a geographical barrier that could have played an important role in the evolution of phenotypic and genetic characters not only because of its radical topography but also because of its arid climatic conditions (Winger and Bates 2015; Hazzi et al. 2018; Linares Pal-

omino 2018) which differ greatly from the climate in the adjacent humid montane forests and grasslands (Winger and Bates 2015) where *Phrynopus apumantarum* occurs.

Indeed, most *Phrynopus* species inhabit puna grasslands (Lehr et al. 2005; Chávez et al. 2015; Lehr and Rodríguez 2017; Rodríguez and Catenazzi 2017). Despite other species having been recorded in two or three localities (von May et al. 2017), *P. apumantarum* sp. nov. seems to be restricted to a single place. Our field efforts sampling multiple sites were unsuccessful, suggesting a high level of micro-endemism, that could be related to their poor locomotor skills. As previously proposed (Chávez et al. 2020), we consider the Nudo de Pasco in Central Peru as the main diversity hotspot for the genus. The type locality of *P. apumantarum* sp. nov. is the southernmost locality from Nudo de Pasco among all recorded *Phrynopus* species. In order to understand how the genus was able to extend its distribution that south, we suggest that further studies focusing on the role played by Nudo de Pasco and adjacent areas are necessary to explore hypotheses about the diversification and distribution patterns of this taxon.

Moreover, the westernmost locality recorded for the genus corresponds to *Phrynopus thompsoni* (Duellman 2000), a species that is only known for its type locality in the Pacific drainage (08°00'S, 78°28'W), far western than any other *Phrynopus* species, and the only species reaching the Andean Pacific slopes. Additional specimens or information about *P. thompsoni* has not been reported since its description. Thus, due its disjunctive distribution and unknown phylogenetic relationships, we suggest that further genetic analysis should focus on *P. thompsoni* and clarify its taxonomic status.

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References

- Benham PM, Witt CC (2016) The dual role of Andean topography in primary divergence: functional and neutral variation among populations of the hummingbird, *Metallura tyrianthina*. *BMC Evolutionary Biology* 16(1): 22. <http://doi.org/10.1186/s12862-016-0595-2>
- Chávez G, Santa-Cruz R, Rodríguez D, Lehr E (2015) Two new species of frogs of the genus *Phrynopus* (Anura: Terrarana: Craugastoridae) from the Peruvian Andes. *Amphibian and Reptile Conservation* 9: 15–25.
- Chávez G, García-Ayachi LA, Catenazzi A (2020) A new species of frog (Terrarana, Strabomantidae, *Phrynopus*) from the Peruvian Andean grasslands. *PeerJ* 8: e9433. <https://doi.org/10.7717/peerj.9433>
- De la Riva I, Chaparro JC, Castroviejo-Fisher S, Padial JM (2017) Underestimated anuran radiations in the high Andes: five new species and a new genus of Holoadeninae, and their phylogenetic relationships (Anura: Craugastoridae). *Zoological Journal of the Linnean Society* 182: 129–172. <https://doi.org/10.1093/zoolinnean/zlx020>
- Duellman WE (1999) Patterns of distribution of amphibians, a global perspective. Baltimore, Maryland: Johns Hopkins University Press. 648 pp. <https://doi.org/10.56021/9780801861154>
- Duellman WE (2000) Leptodactylid frogs of the genus *Phrynopus* in northern Peru with descriptions of three new species. *Herpetologica* 56(3): 273–285.
- Duellman WE, Lehr E (2009) Terrestrial-breeding Frogs (Strabomantidae) in Peru. Münster: Natur und Tier Verlag, 382 pp.
- Finer F, Novoa S, Snelgrove C (2015) Deforestation hotspots in the Peruvian Amazon. MAAP: 26. Monitoring of the Andean Amazon Project. <https://maaproject.org/2016/hotspots2015/> [Accessed on 8th Sept. 2022]
- Frost DR (2023) Amphibian Species of the World: an Online Reference. Version 6.1. New York: American Museum of Natural History. [Accessed 21 February, 2023]
- Gregory-Wodzicki KM (2000) Uplift history of the Central and Northern Andes: a review. *Geological Society of America Bulletin* 112: 1091–1105. [https://doi.org/10.1130/0016-7606\(2000\)112%3C1091:UHOT-CA%3E2.0.CO;2](https://doi.org/10.1130/0016-7606(2000)112%3C1091:UHOT-CA%3E2.0.CO;2)
- Hazzi NA, Moreno JS, Ortiz-Movliav C, Palacio RD (2018) Biogeographic regions and events of isolation and diversification of the endemic biota of the tropical Andes. *Proceedings of the National Academy of Sciences of the United States of America* 115(31): 7985–7990. <https://doi.org/10.1073/pnas.1803908115>
- Hedges SB, Duellman WE, Heinicke MP (2008) New World direct-developing frogs (Anura: Terrarana): Molecular phylogeny, classification, biogeography, and conservation. *Zootaxa* 1737: 1–182. <https://doi.org/10.11646/zootaxa.1737.1.1>
- Heinicke MP, Lemmon AR, Lemmon EM, McGrath K, Hedges SB (2017) Phylogenomic support for evolutionary relationships of New World Direct-developing frogs (Anura: Terrarana). *Molecular Phylogenetics and Evolution* 118: 145–155. <https://doi.org/10.1016/j.ympev.2017.09.021>
- Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* 30(4): 772–780. <https://doi.org/10.1093/molbev/mst010>
- Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution* 35: 1547–1549. <https://doi.org/10.1093/molbev/msy096>
- Landauro CZ, Garcia-Bravo A, Venegas PJ (2015) An endemic new species of *Ameiva* (Squamata: Teiidae) from an isolated dry forest in southern Peru. *Zootaxa* 3946(3): 387–400. <https://doi.org/10.11646/zootaxa.3946.3.6>
- Lehr E, Moravec J, Cusi JC (2012) Two new species of *Phrynopus* (Anura, Strabomantidae) from high elevations in the Yanachaga-Chemillén National park in Peru (Departamento de Pasco). *ZooKeys* 235: 51–71. <http://doi.org/10.3897/zookeys.235.3885>
- Lehr E, Frittsch G, Müller A (2005) Analysis of Andes frogs (*Phrynopus*, Leptodactylidae, Anura) phylogeny based on 12S and 16S mitochondrial rDNA sequences. *Zoologica Scripta* 34: 593–603. <https://doi.org/10.1111/j.1463-6409.2005.00212.x>
- Lehr E, Rodríguez D (2017) Two new species of Andes Frogs (Craugastoridae: *Phrynopus*) from the Cordillera de Carpish in central Peru. *Salamandra* 55(3): 327–338.
- Lehr E, von May R, Moravec J, Cusi JC (2017) A new species of *Phrynopus* (Amphibia, Anura, Craugastoridae) from upper montane forests and high Andean grasslands of the Pui Pui Protected Forest in central Peru. *ZooKeys* 713: 131–157. <http://doi.org/10.3897/zookeys.713.20776>
- Lynch JD, Duellman WE (1997) Frogs of the genus *Eleutherodactylus* in western Ecuador. Systematics, ecology, and biogeography. Lynch: The University of Kansas Special Publication 23: 1–236. <https://doi.org/10.5962/bhl.title.7951>
- Mamani L, Malqui S (2014) A new species of *Phrynopus* (Anura: Craugastoridae) from the central Peruvian Andes. *Zootaxa* 3838: 207–214. <https://doi.org/10.11646/zootaxa.3838.2.5>
- Nguyen LT, Schmidt HA, Haeseler A, Minh BQ (2015) IQ-TREE: A fast and effective stochastic algorithm for estimating maximum likelihood phylogenies. *Molecular Biology and Evolution* 32: 268–274. <https://doi.org/10.1093/molbev/msu300>
- Padial JM, Grant T, Frost D (2014) Molecular systematics of Terraranas (Anura: Brachycephaloidea) with an assessment of the effects of alignment and optimality criteria. *Zootaxa* 3825(1): 1–132. <https://doi.org/10.11646/zootaxa.3825.1.1>
- Rodríguez LO, Catenazzi A (2017) Four new species of *Phrynopus* (Anura: Terrarana: Craugastoridae) from Peru. *Zootaxa* 4273: 381–406. <https://doi.org/10.11646/zootaxa.4273.3.4>
- Santos JC, Coloma LA, Summers K, Caldwell JP, Ree R, Cannatella DC (2009) Amazonian amphibian diversity is primarily derived from late Miocene Andean lineages. *PLOS Biology* 7(3): e1000056. <https://doi.org/10.1371/journal.pbio.1000056>
- Ttito A, Landauro CZ, Venegas PJ, De la Riva I, Chaparro JC (2016) A new species of *Telmatobius* Wiegmann, 1834, from the eastern Cordillera Central of the Andes, Perú (Anura: Telmatobiidae), with description of its tadpole, and range extension of *T. mendelsoni* De la Riva et al., 2012. *Annals of Carnegie Museum* 83(4): 255–268. <https://doi.org/10.2992/007.083.0402>
- Venegas PJ, Echevarría LY, García-Ayachi LA, Landauro CZ (2020) Two new sympatric species of *Stenocercus* (Squamata: Iguania) from the

inter-Andean valley of the Mantaro River, Peru. Zootaxa 4858(4): 555–575. <https://doi.org/10.11646/zootaxa.4858.4.5>

von May R, Lehr E, Rabosky DL (2018) Evolutionary radiation of earless frogs in the Andes: molecular phylogenetics and habitat shifts in high-elevation terrestrial breeding frogs. PeerJ 6: e4313. <https://doi.org/10.7717/peerj.4313>

Wiens JJ (2007) Species delimitation: new approaches for discovering diversity. Systematic Biology 56(6): 875–878. <https://doi.org/10.1080/10635150701748506>

Winger BM, Bates JM (2015) The tempo of traits divergence in geographic isolation: avian speciation across the Marañón Valley of Peru. Evolution 69: 772–787. <https://doi.org/10.1111/evo.12607>

Appendix I

Material examined

Phrynopus anancites— PERU: LA LIBERTAD: Ventanas (8°01'53.54"S, 77°24'28.06"W, 3820 m), MUSM 33168 (holotype).

Phrynopus bracki— PERU: PASCO: 2.9 km N, 5.5 km E (airline) Oxapampa, Cordillera Yanachaga (2600 m), USNM 286918 (holotype), 286919, MUSM 4400 (paratype).

Phrynopus capitalis— PERU: LA LIBERTAD:: Lake Manachahui (7°41'47"S, 77°30'56"W, 3600 m), AMNH 134158 (holotype), MUSM 8959 (paratype).

Phrynopus chaparroi— PERU: JUNÍN: Canchamalca, Distrito de Comas, Provincia Concepción, (11°44'45"S, 74°58'47"W): MHNC 10983 (holotype, 4490 m), MHNC 10980–10982 (4490 m), MHNC 10984 and MHNC 10985 (4205 m).

Phrynopus dagmarae— PERU: HUÁNUCO: Distrito Chaglla, Provincia Pachitea, Chaglla-Paltenanan (09°51'27"S, 75°53'12"W): SMF 80480–83; Maraypata (10°09'35"S, 7°6'06'05"W, 3370 m), SMF 80475 (paratype); Palma Pampa (09°53'12"S, 75°53'22"W): SMF 80476–79 and 80629–33 (paratypes); E slope Cordillera Carpish, Carretera Central, 2400 m: KU196592.

Phrynopus heimorum— PERU: HUÁNUCO: Provincia Ambo, Conchamarca: SMF 80470, 80474 (09°59'44"S, 76°09'40"W, 3420m); 500 m east of Conchamarca (09°59'44"S, 76°09'40"W, 3420 m): SMF 80471–80472.

Phrynopus horstpauli— PERU: HUÁNUCO: Provincia Ambo, 7 km east of Conchamarca, Huacamonte forest (09°59'55"S, 76°09'43"W, 3070 m): SMF 80447–52, 80459–60; Huacamonte forest (09°59'41"S, 76°09'44"W, 3420 m): SMF 80466–67; Ichocan, Jatunloma, 3100 m: KU 291399–291400, 311452: 10 km E of Conchamarca, 3420 m: KU 311453.

Phrynopus kauneorum— PERU: HUÁNUCO: Chaglla, SMF 80626–28, SMF 80484–85, AMNH 311451.

Phrynopus montium— PERU: JUNÍN: Tarma, 45 min. of Maranyioc (12000 ft = 3658 m), USNM 217416–17; JUNÍN: Prov. Yauli, 9.5 min. from La Oroya (13000 ft = 3962 m), AMNH 84795.

Phrynopus remotum— PERU: HUANUCO: Marañón Province: Villa Rica de Chona, on the trail to Antaquero Community (8°43'38.2"S, 76°59'25.95"W; 3,730 m a.s.l.): CORBIDI 20531–33.

Phrynopus valquii— PERU: SAN MARTÍN: Río Abiseo NP: MUSM 3824, MUSM 3821, 3823, KU 220918 and AMNH 134154–55.

Appendix II

Table A1. GenBank accession numbers for the taxa and genes sampled in this study. New sequences produced for this study (*P. apumantarum*, CORBIDI 20438) are in bold.

Taxon	16S	12S	COI	RAG1	Tyr	Voucher #
<i>Niceforonia brunnea</i>	EF493357	EF493357	na	EF493422	EF493484	KU178258
<i>Lynchius flavomaculatus</i>	EU186667	EU186667	na	EU186745	EU186766	KU218210
<i>Lynchius nebulanastes</i>	EU186704	EU186704	na	na	na	KU181408
<i>Lynchius oblitus</i>	KX470782	KX470775	na	KX470791	na	MHNC8652
<i>Lynchius parkeri</i>	EU186705	EU186705	na	na	na	KU181307
<i>Lynchius simmonsii</i>	JF810004	JF809940	na	JF809915	JF809894	QZ41639
<i>Lynchius tabaconas</i>	KX470780	KX470773	na	na	KX470796	MHNC8637
<i>Oreobates amarakaeri</i>	JF809996	JF809934	na	JF809913	JF809891	MHNC6975
<i>Oreobates ayacucho</i>	JF809970	JF809933	na	JF809912	JF809890	MNCN_IDIR5024
<i>Oreobates cruralis</i>	EU186666	EU186666	na	EU186743	EU186764	KU215462
<i>Oreobates gemcare</i>	JF809960	JF809930	na	JF809909	na	MHNC6687
<i>Oreobates granulosus</i>	EU368897	JF809929	na	JF809908	JF809887	MHNC3396
<i>Phrynopus apumantarum</i>	OQ559663	OQ557735	OQ578991	OQ578990	OQ556799	CORBIDI20438
<i>Phrynopus auriculatus</i>	EF493708	EF493708	na	na	na	KU291634
<i>Phrynopus auriculatus</i>	MF186348	MF186290	MF186466	na	MF186582	MUBI 6471
<i>Phrynopus badius</i>	MG896572	MG896595	MG896612	MG896619	na	MUSM31099

Taxon	16S	12S	COI	RAG1	Tyr	Voucher #
<i>Phrynopus barthlenae</i>	MF186350	MF186292	MF186464	na	na	MHNSM20609
<i>Phrynopus bracki</i>	EF493709	EF493709	na	EF493421	na	USNM286919
<i>Phrynopus bufoides</i>	AM039645	AM039713	na	na	na	MTD45072
<i>Phrynopus daemon</i>	MG896574	MG896597	na	na	na	MUSM32747
<i>Phrynopus heimorum</i>	AM039635	AM039703	MF186462	MF186545	MF186580	MTD45621
<i>Phrynopus horstpauli</i>	MF186364	MF186303	na	na	MF186584	MTD44335
<i>Phrynopus interstinctus</i>	MG896575	MG896598	MG896614	MG896621	na	MUSM29543
<i>Phrynopus inti</i>	MF651906	MF651913	na	MF651918	MF651921	UMMZ245218
<i>Phrynopus juninensis</i>	MF651908	MF651915	na	MF651920	na	MUSM33258
<i>Phrynopus kauneorum</i>	AM039655	AM039723	na	na	na	MHNSM20595
<i>Phrynopus miroslawae</i>	MF186393	MF186312	MF186463	MF186542	MF186585	MUBI 6469
<i>Phrynopus montium</i>	MG896579	MG896602	na	MG896625	na	MUSM33260
<i>Phrynopus peruanus</i>	MG896582	MG896605	MG896615	MG896626	MG896631	MUSM38316
<i>Phrynopus pesantesi</i>	AM039656	AM039724	na	na	na	MHNSM19860
<i>Phrynopus remotum</i>	MT261899	na	MT263073	MT431670	MT431667	CORBIDI20531
<i>Phrynopus remotum</i>	MT261774	na	MT434010	na	MT431668	CORBIDI20532
<i>Phrynopus remotum</i>	MT261773	MT272829	MT434009	MT431671	MT431669	CORBIDI20533
<i>Phrynopus</i> sp	AM039657	AM039725	na	na	na	MTD45075
<i>Phrynopus spl</i>	MG896589	MG896606	na	MG896629	na	MUSM33261
<i>Phrynopus tautzorum</i>	AM039652	AM039720	na	na	na	MHNSM20613
<i>Phrynopus tribulosus</i>	MF186424	MF186330	MF186467	MF186547	MF186579	MUBI 7166
<i>Phrynopus unchog</i>	MG896591	MG896608	na	na	na	MUSM32748
<i>Phrynopus vestigiatus</i>	MG896593	MG896610	MG896617	na	na	MUSM29542

Supplementary material 1

High resolution photographs of type specimens

Authors: Germán Chávez, Luis A. García-Ayachi, Alessandro Catenazzi

Data type: pdf file

Explanation note: 1,2) Holotype CORBIDI 20533 in profile (1=right side, 2=left side). 3) Paratype CORBIDI 20439 in profile (right side). 4) Paratype CORBIDI 20437 in profile (right side). 5) Paratype CORBIDI 20432 in profile (right side). 6) upper palate area of paratype CORBIDI 20432, 7) upper palate area of paratype CORBIDI 20437. 8) upper palate area of paratype CORBIDI 20439

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